Dear Editor of World Journal of Stem Cell

We would like to thank editors of World Journal of Stem Cell and unknown reviewers of our manuscript for carefully reading our manuscript and providing comments. We have addressed the concerns of the respected editor and reviewers as follows. In addition, we have revised the manuscript according to *Format for Manuscript Revision: Letters to the Editor* and *Guidelines and Requirements for Manuscript Revision: Letters to the Editor*.

It is worth mentioning that in the revised manuscript, the updated text appears in blue colour.

Kind Regards

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Answering to Reviewer #1:

1. Clear recommendations arising from the lessons learned from this case study should be included for the benefit of scientists and clinicians working in this field.

A sentence has been added as "The significance of identification of the initiating factors and recognition of the pathogenic mechanism lies in that which would be helpful for doctors to select an optimal therapeutic approach and to improve the treatment outcome, not only targeting immune-mediated mechanisms as the generally accepted first-line ISTs but also taking into account the initiating factors and the sustaining forces." to highlight the importance of recognizing the pathogenic mechanism (Reflected in page 14, line 14 to 19).

2. Line 4: "…by theses unwanted…"

The sentence has been revised as "An inappropriately enhanced inhibitory and pro-apoptotic capacity of these deranged immune reactions to HSPCs from highly sensitized "autoreactive" cytotoxic T lymphocytes (CTLs) by targeting against yet unidentified antigens is responsible for the overall pathophysiology."(Reflected in page 5, line 6 to 10)

3. Line 11: "…mechanisms imposed on HSPCs" instead of imposing.

This sentence has been revised as "Despite the absence of direct evidences in the present case due to the lack of more complex and elegant laboratory investigations, the emergence of MCH following the settlement of myelosuppression raises the intriguing possibility that immunogenic neo-epitopes on genetically damaged hematopoietic cells caused by either genotoxic agents or by spontaneously mutated genes may become the directly targeted antigens of host's potential immunological surveillance on malignant cells and may thereby induce a proinflammatory niche in the BM[14-18,21-23], one of the consequence of which results in a severe hypoplastic BM via the

chronic overproduction of proinflammatory mediators superimposed upon non-targeted neighbouring inculpable cells[4-6,40] but yet probably without clear evidence of dysmorphic features."The word "imposing on" has been replaced by "superimposed upon" (Reflected in page 12, line 13 to 24).

4. Line 38: "Referances:"

The word "Referances:" has been replaced by "References:"

5. Figure 3 figure legend: The word proposition needs to be replaced (proportions?).

The word "proposition" has been replaced by "percentage".

Answering to Reviewer #2:

1. My main concern regarding this manuscript is in its structure. The whole text is presented in a single body text. My opinion is that the whole manuscript should be structured into specific sections, depending on the journal requirements.

The manuscript has been revised according to *Format for Manuscript Revision: Letters to the Editor* and *Guidelines and Requirements for Manuscript Revision: Letters to the Editor*.

2. it would be much better if the presented laboratory results are ordered into tables.

The presented laboratory results have been ordered in a table (Table 1) according to the reviewers' comment. Other useful laboratory information is also listed in this table. Because the results of flow cytometric analysis are already listed in the table, the Figure 3 is presented as a supplementary datum (Supplementary Figure 1) .

3. No abstract was provided, thus it was not reviewed.

We have added an abstract of 209 words (Reflected in page 3, line 1 to 22) and a

core tip of 97 words (Reflected in page 4, line 1 to 11) according to *Guidelines and Requirements for Manuscript Revision: Letters to the Editor*.

Answering to Reviewer #3:

1. Major Comment: While the description is adequate, there is little direct evidence for the proposed pathogenetic mechanism (namely that bone-marrow suppression is an antineoplastic defense) in the clinical data. Interpretation of the patient's data should be done with great caution, because this patient received a very large number of distinct treatments over many years, each one of which may have contributed to this unfavorable outcome.

First, in order to easily express the bystander effect in the suppression of normal hematopoiesis, we have added sentences to address the proinflammatory mediators being the direct bone marrow suppressive effector. These sentences are "Overproduction of soluble proinflammatory mediators by phenotypically and functionally skewed CTLs, mainly through expressing and secreting high levels of interferon -γ (IFN-γ) and tumor necrosis factor -α (TNF-α), with an IFN-γ predominance, is the distinctive immunological feature. it is the inflammatory cytokines that upregulate the expression of pattern recognition receptors (PRRs), antigen-presenting and apoptosis-associated molecules on hematopoietic precursors, and exert the direct detrimental effect on normal hematopoiesis in a manner resembling bystander effect[4-6]."(Reflected in page 5, line 12 to 20).

Second, we have described the evidence as an indirect information. It has been revised as "The emergence of MCH in a patient with refractory severe aplastic anemia (RSAA) following the resolution of BMS by treatment of his gut inflammatory disease with gut-cleansing preparations (GCP) provides an indirect but forceful information in support of this extrapolation."(Reflected in page 6, line 19 to 22).

Third, we have addressed his genotoxic exposure and the associated

hematological diseases. These points presented as (1) "The particularly noteworthy event was the dimethylbenzene exposure due to his house being decorated 8 months before the disease onset."(Reflected in page 6, line 30 to page 7, line 2), (2) "Dimethylbenzene is a well-known genotoxic agent. In addition to AAA, the genotoxicity of dimethylbenzene exposure has been recognized to be closely linked to the pathogenesis of MDS and acute leukemia as well[1,39]."(Reflected in page 11, line 16 to 18).

Fourth, we think it is impossible that the development of an myeloproliferative neoplasm occurred in such a short time, and the recurrences of myelosuppression in the one year of intermittent GCP treatments strongly indicates the immunosuppressive effect on clonal hematopoiesis. These points are addressed as "Although clonal evolution frequently develops due to the selective pressure in the context of severe immunological attack and gradual loss of HSPCs, and may be responsible for the progression and exacerbation of impaired hematopoietic capacity as evidenced by the gradual loss of sensitivity to CsA treatment, it is unconceivable that the gene damages caused by these spontaneous mutations were accumulated so enriched that they are sufficient to induce malignant transformation within only four months, meaning that the oncogenic genes had pre-existed, most probably initiated by the genotoxicity of dimethylbenzene exposure preceding the onset of AAA and precipitated by subsequently spontaneous mutations and selective pressure. Furthermore, the 23 years history of hypoplastic and fat-replaced BM, several recurrences in the intermittentence of GCP treatments (indicating the resilience of BMS), and the emergence of MCH that occurred only after the successive administration of rifampicin and berberine with monthly administered GCP treatments, also suggest that the immunogenic antigens on HSPCs had pre-existed for a very long time."(Reflected in page11, line 20 to page 12, line 5).

Fifth, we have cited an elegant experimental research in which a spontaneously mutated gene is the direct target of immune attack, and the

development of immune-mediated bone marrow failure that morphologically and immunologically resembled aplastic anemia occurred with an increased production of proinflammatory mediators. This experimental research is generalized as "Xin, et al.[40] established an animal model of AAA by using *Mx1Cre*$^{+}$*Tak1*$^{fx/fx}$ (*Tak1*^{mut}) mice in which the gene encoding *TGFβ-activated kinase-1 (Tak1)* could be spontaneously mutated in a small subset of hematopoietic cells and the suppressive effect on proliferation and differentiation merely occurred in mutated cells. In this animal model, high levels of IFN-γ and TNF-α were identified, and the characteristic AAA morphology developed with a necroptotic manner of cell death, suggesting that BMS could be caused by the bystander insult that was originated from the genetically damaged hematopoietic cells." (Reflected in page 12, line 5 to 13)

Sixth, we have compared the cellular immune-mediated bone marrow failure (aplasic anemia, hypoplasic myelodysplastic syndrome and a subgroup of low risk myelodysplastic syndrome that responds to immunosuppressive therapy) with classic myelodysplastic syndrome. Their common pathogenic basis is the genetically damaged hematopoietic precursors. However, because the different mechanism behind the development of bone marrow failure, significant differences in morphology and immunology are presented between these disorders. This means that the immunogenic products of mutated genes can become the target of autoreactive cytotoxic T cells. This point are addressed as "This cellular immune-mediated mechanism characterized by IFN-γ-predominated functional inhibition, commonly in a way of apoptosis or necroptosis, of autologous hematopoiesis, also probably playing a fundamental role in the pathogenesis of hMDS and a subgroup of low risk MDS, is completely distinct from the pathogenic mechanisms in classic MDS that is characterized by the hypercellular BM and ineffective hematopoiesis, in which the innate immune responses were directly elicited

by PRRs sensing damage-associted molecular patterns (DAMPs) in the genetically damaged hematopoietic precursors with the features of increased proliferative activity of clonal hematopoiesis, inflammosome formation, cell death in a manner of pyroptosis, TNF-α-predominance and effective lenalidomide treatment[46-48]."(Reflected in page 14, line 3 to 14).

Finally, we have drawn a summery as "Overall, the extrapolation is only an indirect implication from the treatment process of a RSAA patient, and further investigations are critically needed to look for the direct evidence and to illustrate the precise mechanism." to point out that the hypothesis is an indirect implication, and it need extensive investigations (Reflected in page 14, line 19 to 22).

2. Minor comments. "driver of the deranged autoimmunity" - I suggest to change to "the driving force behind the autoimmune disorder".

The sentence has been revised as "Recently, much like in other autoimmune diseases[10], the driving force behind the initiation, development, chronicity and progression of AAA pathophysiology has been proposed to come from the altered composition of gut microbiota and the compromised integrity of intestinal barrier[11-13]." (Reflected in page 6, line5 to 9)

3. "A reduced ratio of CD4+/CD8+ cells confirmed the cellular immune-mediated response [1,2]". This sentence is unclear. Why should a reduced ratio of CD4+/CD8+ cells provide evidence of cellular immune-mediated response, and against what antigens would this response be directed?

The sentence has been revised as "A reduced ratio of CD4+/CD8+ cells confirmed the CD8+ CTL-predominated immune responses in excellent concordance with the characteristic immunological profile in AAA[1,2] and other cellular immune-mediated autoimmune diseases, while an increased percentage of CD3+CD5+ cells reinforced the autoimmune nature. An

enhanced ratio of CD33+/CD19+ cells and an increased percentage of CD14+ cells, CD3-CD56+ cells reflected a bias of the homeostatic hematopoiesis towards innate immune responses against ongoing infections[27,28]." (Reflected in page 9, line 4 to 11)

4. I found the photomicrographs not very useful. The reader might be helped by the inclusion of arrows pointing to the cells specifically bearing features of interest referred to in the text.

We have drawn arrows on the related tissues and cells in corresponding pictures, and reorganized the figure legends.

Answering to Science editor

1. The highest single-source similarity index in the CrossCheck report showed to be 7%. Please rephrase these repeated sentences.

We have rephrased the sentences that we previously used to describe the reported patient.

2. I found no "Abstract" section. Please write this section.

We have added an abstract of 209 words (Reflected in page 3, line 1 to 23) and a core tip of 97 words (Reflected in page 4, line 1 to 11) according to *Guidelines and Requirements for Manuscript Revision: Letters to the Editor*.

3. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

We have arranged the figures in a ppt file.

In addition to answering the reviewers, we have some revision to explain. We all think it is very helpful for understanding and interpreting the laboratory investigation and therapeutic outcome in this manuscript.

1. We all think that the title of this manuscript would be better revised as "*Acquired aplastic anemia: is bystander insult to autologous hematopoiesis driven by immune surveillance on malignant cells?*". This title may be more succinct,

attractive and easily-understood, and directly points out the hypothesis is derived from a complicated treatment process in a patient with refractory severe aplastic anemia.

2. The words in this manuscript have been expanded from 1500 to 3000, and the cited references have been expanded from 18 to 48, in an attempt to better illustrate the hypothesis based on the established researches.